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(57) Abstract

Methods have been developed for protection of structures from termite infestation. In the preferred embodiment, termites are attracted into contact with a potentially lethal fungus, such as *Metarhizium anisopliae* or *Beauveria bassiana*, using an attractant, such as the fungus *Gloeophyllum trabeum* or its volatile products. In another embodiment, a fungus is used which is naturally repellent to the termites, such as *M. anisopliae* and serves to prevent termites from entering into an area bounded by the repellent fungus. The entomopathogenic fungus, alone or in combination with an attractant fungus, is provided either in the form of an infection chamber or formulated with a nutrient composition and administered directly to inhabited galleries or to the termite nest to initiate infection, transmission and colony mortality. Examples demonstrate control of termites using chambers containing *M. anisopliae* and *B. bassiana*.

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BIOLOGICAL CONTROL OF TERMITES

Background f the Inv ntion

The present invention is generally in the field of biological control of insect pests, specifically in the area of the use of a behavioral modifier in combination with an entomopathogenic fungus for the control of termites.

Termites cause major economic loss through destruction of wooden structures, including residences, commercial buildings, and furniture, and, by deposit of earth and clay during the infestation process. Termites also damage carpet and other floor coverings, wall coverings, plaster, siding, and dry wall materials. Infestation of a residence with termites is a particular nuisance for homeowners, especially those who are trying to sell their houses.

The most common means of termite control is a periodic spraying with chemical insecticides or injection of soil surrounding structures with large quantities of insecticides. The problems with chemical insecticides are that they are expensive; their long term effects on people, pets, and the environment are unknown; resistant insect strains tend to develop over time following repeated chemical treatment, requiring increasingly larger quantities or the application of different chemicals; and the chemicals currently used tend to repel termites rather than kill them and thus are less effective in termite control than is presumed.

Insect pathogens are a possible alternative to the common use of highly toxic chemical insecticides for the control of insect pests. Fungi are one promising group of insect pathogens suitable for use as biological agents for the control of termites.

Fungi are found either as single cell organisms or as multicellular colonies. They are eukaryotic and therefore more highly differentiated than bacteria but

less differentiated than higher plants. Fungi are incapable of using light as an energy source and thus are restricted to a saprophytic or parasitic existence. The most common mode of growth and reproduction for fungi is vegetative or asexual reproduction, which involves sporulation followed by germination of the spores. Asexual spores, or conidia, form at the tips and along the sides of hyphae, the branching filamentous structures of multicellular colonies. In the proper environment, the conidia germinate, become enlarged, and produce germ tubes. The germ tubes develop over time into hyphae that in turn form colonies.

To date, the majority of work evaluating entomopathogenic fungi for biological control of insect pests has focused on applications involving agriculturally important insect pests and mosquitos. *Metarhizium anisopliae* is one of the most widely studied fungi for biological control of insects. *M. anisopliae* has been administered to insect pests by a number of methods, including direct spraying, injection, and application of the fungus to the plant or soil material on or in which the insect lives or feeds. In some insect species, infection with the fungus has been shown to result in death. The fungi *M. anisopliae*, as reported by Hanel and Watson, 73 Bull. Ent. Res. 305-313 (1983), and *Beauveria bassiana*, as reported by Bao and Yendol, 16 Entomophaga 343-352 (1971), are examples of fungi that infect termites. In one species of termites, infected individuals transmitted the fungus to non-infected members of their colony, as reported by Kramm *et al.*, 40 J. Invert. Pathol. 1-6 (1982).

The limitation of the majority of the prior research using fungal pathogens of insects is that it has been conducted under laboratory conditions that are quite different from the conditions under which

the insects are actually found. In most reported cases, death of the treated insects was achieved by ingestion or injection of very large quantities of spores, which may be toxic in and of themselves. In other cases, infection was achieved by rolling or dusting the insect in a test tube or petri dish containing large quantities of fungal spores. It is clearly impractical to use such methods commercially or on a large scale. Moreover, government regulations would make it difficult to register a fungal insecticide that requires the random release of large quantities of fungal spores in areas of insect infestation, particularly where people or food could be contaminated. No one has yet developed a consistent and commercially viable way of infecting termites and assuring that the fungal inoculum is widely dispersed, especially through the breeding population, for the management and biological control of termites infesting houses or buildings.

U.S. Patent Nos. 5,057,315 and 5,057,316 to Gunner et al., disclose the use of an infection chamber containing a fungal pathogen to attract and lethally infect insects with the fungus. Examples include cockroaches and flying insects. The infection chamber design both maintains the fungal culture and forces the insect into contact with the fungus. Insects are attracted to the chamber by anyone or more of such means as the shape of the chamber, the use of food, attractants, flavorings, scents, or colors, depending on the insect type.

It would be desirable to provide an infection chamber for termites. However, such a chamber has not previously been described and it is unclear what shape such a chamber would have nor what would be used as the attractant for the termites.

It is therefore an object of the present invention to provide a method and apparatus for the

biological control of termites by the use of entomopathogenic fungi.

It is a further object of the present invention to provide a device for the convenient, reliable, and economically feasible application of fungi in the biological control of termites.

It is a further object of the present invention to provide a method and means to prevent termite infestation and damage of a structure or area by repelling the termites.

It is further object of the present invention to provide a method and means for ultimately infecting all or most of the termites in a breeding colony.

It is another object of the present invention to provide a method and means for infection and killing of termites by a variety of fungi so that development of resistant strains is avoided.

Summary of the Invention

Methods have been developed for protection of structures from termite infestation. In the preferred embodiment, termites are attracted into contact with a potentially lethal fungus, such as *Metarhizium anisopliae* or *Beauveria bassiana*, using a recruitment stimulant which evokes a series of behaviors, including feeding, arresting, and trail following (referred to herein as an "attractant"), such as the fungus *Gloeophyllum trabeum* or its volatile products. In another embodiment, a fungus is used which is naturally repellent to the termites, such as *M. anisopliae* and serves to prevent termites from entering into an area bounded by the repellent fungus. The entomopathogenic fungus, alone or in combination with an attractant fungus, is provided either in the form of an infection chamber or formulated with a nutrient composition and administered directly to inhabited galleries or corridors to the termite nest to initiate infection, transmission and colony

mortality. An advantage of the fungi is that, once established, the fungi continue to protect a structure from termites for an indefinite period of time. The two most preferred entomopathogenic fungi are *Metarhizium anisopliae* and *Beauveria bassiana*, although other fungi can be used that are pathogenic when the termite is inoculated via the infection chamber. *M. anisopliae* is the preferred repellent fungus. *Gloeophyllum trabeum* is the preferred attractant fungus. Examples demonstrate control of termites using chambers containing *M. anisopliae* and *B. bassiana*.

Brief Description of the Drawings

Figure 1 is a prospective view of a termite infection chamber.

Figures 2A and 2B are graphs of termite decline in a habit trail after exposure to fungus while foraging, graphing percent survival versus time after treatment in days. Figure 2A is the percent survival after exposure to *M. anisopliae* (squares) versus control (circles) and Figure 2B is the percent survival after exposure to *B. bassiana* (squares) versus control (diamonds).

Detailed Description of the Invention

The present invention is primarily based on two discoveries: (1) that entomopathogenic fungi can be used to infect and kill termites but that an attractant is required to induce the termite into contact with the fungus and (2) that some types of fungi, by virtue of their natural repellency to termites, can be used to repel termites from an area. The pathogenic fungi, alone or in combination with an attractant fungus or compounds derived therefrom, or the repellent fungus can be administered to the area to be treated or protected in a nutrient formulation

or an infection chamber to initiate a fungal culture for infection or repellency of termites.

A major problem associated with entomopathogenic fungi for termite control is that the pathogenic fungi appear to repel the termites, making it extremely difficult to achieve infection sufficiently widespread to significantly reduce the termite population. This problem has been overcome by combining a fungal or other attractant with the entomopathogenic fungus which overcomes the repellency of the entomopathogenic fungus.

Another major problem has been how to sustain a fungal culture which effectively kills the termites. This problem has been overcome by developing a nutrient formulation which is effective in establishing a fungal culture when the formulation is administered to soil containing the termites. The nutrient formulation consists of the fungus on a nutrient medium such as bran or other cellulose based food product which can be administered as a dry powder, such as a cereal grain or sawdust.

Still another major problem has been how to infect the termites with a lethal infection of the fungus. This problem has been addressed through the use of an infection chamber which is effective in establishing fungal cultures in the vicinity of the area to be treated or protected. The infection chamber includes a nutrient source for the fungal culture(s) as well as contains and protects the fungi prior to establishment of the fungi at the site to be protected or treated.

Selection of the Fungi

Useful fungi can be obtained as isolates from infected termites (pathogenic fungi) or from termite infested wood (attractant fungi) or as isolates from soil using conventional techniques or from stored culture collections. These fungi can be obtained from

the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland (ATCC) or USDA-ARS collection of entomopathogenic fungal cultures, U.S. Plant, Soil and Water Lab, Tower Road, Ithaca, New York (ARSEF), where they are available without restriction. Soil isolates are obtained by screening soil samples for the presence of fungi, then testing the fungi in a bioassay for their effect on insects. Pathogenicity is established when the exposed insects die.

At least two species of entomopathogenic fungi, *M. anisopliae* and *B. bassiana*, have been shown to infect and kill termites. Examples include *MM. anisopliae* ATCC 62176 and ARSEF 1112 which have also been shown to repel termites.

Useful fungi can be obtained by screening soil isolates by exposing termites under laboratory conditions to the fungus, as described in the examples below. Useful pathogenic fungus are those that kill the termites following direct contact. Useful repellent fungus are those that repel termites in soil box tests as described below. As used herein, "repellent fungus" includes a fungus such as *Metarhizium spp.*, or compounds produced by the fungus which effectively repel the termites.

Attractants

Attractants that are useful are those that attract termites, or stimulate termites to recruit other colony members to aggregate and feed. Preferred attractants for termites include *Gloeophyllum trabeum* (formerly *Lenzites trabea*) and some of its metabolic products, wood decayed by certain fungi containing extractable attractant substances, and certain organic compounds which mimic trail following pheromones, as described by Watanabe and Casida, 56:3 J. Econ. Ent. 300-307 (1963), Matsumura, F., et al., 65:2, L. Econ. Ent. 600-602 (1972), Smythe, R.V., et al., 58:3 J.

Econ. Ent. 420-423 (1965), and Prestwich, G.D., et al., 10:8 Journal of Chemical Ecology 1201-1217 (1984), the teachings of which are incorporated herein. The shape of the chamber and its composition, as well as the location of the chamber can also be used to attract termites.

Wood decayed by some fungi contains attractants to termites, as described in Matsumura, F., et al., 62:3 J. Econ. Ent. 699-603 (1969); Watanabe and Casida (1963). For example, wood decayed by the brown rot fungus *G. trabeum* contains (S.S.E)-3, 6, 8-dodecatriene-1-ol and other compounds known to recruit termites. Grace, J.K., 92 Proc. Entomol. Soc. Wash. 773-777 (1990) performed behavioral bioassays involving trail-following activity in termites in response to extracts from wood decayed by *G. trabeum* combined with antioxidants. Watanabe and Casida (1963) also found that numerous organic compounds can attract termites. It has been observed that termite attractant activity is influenced by the size of the wood, which must exceed a minimum mass to warrant recruitment.

At least one species of fungus, *G. trabeum*, has been shown to produce substances that attract termites and mask the repellency of pathogenic fungi. Others that should be useful are fungi that decay wood. Several isolates of *G. trabeum* are available from the ATCC; ATCC 13021 has been tested and established to be attractive.

Culture Media for Fungus

Suitable culture media are known that can be used in the chamber. Examples of media known to those skilled in the art and commercially available include potato dextrose agar (PDA), or rice agar for the growth of *Metarhizium* and *Beauveria*. Rice agar consists of 1% dextrose, 1% yeast extract, 5% rice flour, 1.5% agar, and 0.5% 5x Dubois sporulation

salts. The 5x Dubois sporulation salts consist of 15 g $(NH_4)_2SO_4$/1000 ml; 0.30 g $MgSO_4$ $7H_2O$/1000 ml; 0.15 g $MnSO_4H_2O$/1000 ml; 0.0375 g $CuSO_4$ $5H_2O$/1000 ml; 0.0375 g $ZnSO_4$ $7H_2$/1000 ml; and 0.0038 g $FeSO_4$ $7H_2O$/1000 ml. Each salt is completely dissolved before the next salt is added, and the solution is autoclaved.

Other useful culture media are known and can be optimized from those that are known by those skilled in the art. For example, either PDA or simple cellulose materials like sawdust can be used as culture media for the growth of *G. trabeum*.

The Infection Chambers.

As used herein, "chamber" refers to an inoculation chamber used to initiate the infection process within the resident population. The infection chamber (1) concentrates an extremely high concentration of pathogenic fungal inoculum in a very small space, directing entering termites into contact with the spores that infect and kill the termites; (2) contains fungal spores and/or mycelium, resulting in minimal exposure of the environment to the fungi and protecting the fungus from the environment, thereby increasing viability of the culture and minimizing contamination of the fungal culture; and (3) attracts termites to the pathogenic fungi through the presence of termite attractants, such as *Gloeophyllum trabeum* or its volatile products, that overcome the repellant characteristic of the pathogenic fungi, alone or in combination with sawdust or wood chips which serve as a source of food for the termites.

The chamber can be made of a non-biodegradable material, or a biodegradable material, and is designed to establish an infection locus effective against a termite infection.

The small, lightweight infection chambers (or "pegs") are unobtrusive and can be easily placed in locations

of heavy termite infestation, increasing the efficacy of the devices.

Within the chamber, one fungal culture can be used as an attractant agent and a second to provide a continuous supply of infectious spores over a prolonged period of time. These spores attach to the termites and originate germ tubes that penetrate the termite cuticle, resulting in death within a short period of time. The attractants, such as the volatile compounds produced by wood decomposition, can be provided alone or in combination with a wood decomposing fungus such as *Gloeophyllum trabeum*. Although the primary means of infection is by external contact, the termites can also be infected by contact with each other and by ingestion of the spores.

These attractants, alone or in combination with cellulose based materials, which serve as a source of food for the termites, trigger the additional termite behavioral response of trail laying between the chamber and the colony, thereby recruiting other individuals who will become infected and thus transfer the spores to others in the colony.

Once inside the chamber the termite comes in direct contact with the entomopathogenic fungus. The conidia of the fungus attach to the body of the termite. After attachment, the conidia germinate on the insect cuticle, forming germ tubes which penetrate the integument of the termite. The germ tubes continue their penetration until they reach the internal body cavity (hemocoel) of the termite and kill it. After the termite dies, given the appropriate conditions of relative humidity and temperature, the fungal mycelia may sporulate on the body of the termite, and other termites may be infected by exposure to the conidia produced on the dead termite. Exposure of non-infected termites to the spores on the surface of infected termites or

termite cadavers effectively transmits the pathogen. Transmission of spores is enhanced by termite social behaviors, e.g., grooming, communication by contact, transporting and burying cadavers, and exchange of fecal materials, as well as cannibalism. Some termites may also ingest the spores, which can contribute to or cause death of the termite.

The chamber consists of an external housing which forms the basic shape of the chamber and an internal core mixture which fills the housing unit. The chamber housing can be constructed with conventional materials including glass, metal, extrudable or moldable plastic, or wood, but is preferably constructed of a biodegradable cellulose based material such as cardboard, paperboard, wood, pressed resin-woodchip or flakeboard, expanded cellulose, papier maché or peat.

As shown in Figure 1, the chamber 10 should have small openings 12 (2-5 mm in diameter) which are large enough to allow free passage of the termites or have thin walls 14 which would allow termites to easily chew through.

The preferred embodiment is a tubular structure. The shape can range from circle to multipointed polygon in cross section, but a triangular tube is preferable. The length of the chamber should range from 10 to 30 cm, and the side width should range from 3 to 8 cm. The chamber tip 16 is pointed in order to facilitate placement of the chamber into the ground. The tip 16 can be made from the same material as the housing walls 14, and coated with resin or other materials to increase strength, or the tip can be made of a harder material such as plastic or wood. The top 18 of the chamber housing is covered with a paper or plastic cap after filling.

Inside the housing, the core of the chamber can be filled with a mixture of several components: (1) a

basic food source for the termites (a cellulose base material, most inexpensively sawdust); (2) the attractant: a 4-8 week old culture of *G. trabeum* grown on sawdust, a killed culture of *G. trabeum*, or extracts of *G. trabeum* cultures, or synthesized chemical attractants, arrestants, pheromones or kairomones; and (3) the lethal agent (*M. anisopliae* or other entomopathogenic organisms growing on solid or liquid medium, formulated wet or dry with food sources and protectants, or spores). The core mixture should contain excess attractant in proportion to lethal fungus, at a preferred ratio of 100 g sawdust inoculated with *G. trabeum*: 5 g dried formulated *M. anisopliae* hyphae mixed with oat bran: 1 g *M. anisopliae* spores.

These components can be spatially separated or mixed uniformly to maximize termite contact with the lethal fungus and to provide an excess dose of the lethal fungal spores to an individual termite. Cultures can be applied to an inert lattice or grid which serves as a support matrix, for growing or positioning the appropriate fungal cultures or for holding nutrients within the device.

The chamber can be appropriately placed by (1) directly forcing it into the ground if the chamber is constructed of a hardened material; (2) forcing it into a hole dug by a tool such as a trowel or core sampling device; (3) placing it into the inner sleeve of a hollow tool which would be used to insert the chambers into the ground.

The following are general guidelines which may be modified depending on the terrain, density and strain of termites, the climate, and other variables which may affect chamber efficacy. Chambers are placed in the perimeter around the area where a structure is to be protected, at a distance of not less than 3.0 meters away but preferably 5 to 10 meters away from

the structure. Chambers should be concentrated in an area with termite activity or between the termite active area and the area to be protected. The chambers should be separated by a distance of 0.1 meter to 1 meter.

Greenhouse and laboratory tests indicate that the lethal fungus *M. anisopliae* remains present in the soil at high levels for more than 90 days, and that *B. beauveria* remains in agricultural soils from at least May to October.

Preparation of the Infection Chamber and Formulation.

An infection chamber suitable for infecting termites can be constructed by initially building a housing unit, as demonstrated in Figure 1, and then filling it with core contents. The housing unit can be cut from flexible flat sheets of cellulose products, folded to the desired shape and glued together. It may be more desirable to mold the sheets first from a plastic, resin/wood, papier maché or peat before cutting and folding. Molding forms the grooves and holes in the walls and folding forms the final shape. It is possible to mold the complete shape at one time or to mold sections which can also be assembled by glue, pins in holes or tabs in slots.

The assembled housing is next filled with the core mixture. The core mixture is formulated in three stages. In Stage I, a cellulose based nutriment, typically sawdust, is prepared for both termites and *G. trabeum*. The sawdust is sterilized by autoclaving twice, in plastic bags or other containers, with a 12-24 hour cooling period between runs. The sawdust is inoculated with *G. trabeum* mycelium harvested from a liquid culture medium such as potato dextrose broth. The sawdust and *G. trabeum* mycelium are mixed and incubated at 24-27°C for 4 to 8 weeks.

In Stage II the cellulose *G. trabeum* culture is mixed with formulated *M. anisopliae* hyphae. This formulation is prepared by mixing *M. anisopliae* hyphae, harvested from liquid culture (1% yeast extract, 1% peptone, 4% dextrose), with a dry food source (oat bran flour), mineral salts and protectants. The mixture is dried and ground to form granules.

Finally, in Stage III, the above preparation is mixed with *M. anisopliae* conidia which are harvested from rice agar growth plates or bags of inoculated rice grains after one to three weeks of growth. The housing is then filled by hand or by machine with the core mixture and sealed with a cap. Alternatively, a support matrix core or grid core can be inserted into the housing. The matrix core has pockets filled with fungal preparations as previously described: *G. trabeum* on a cellulose base and *M. anisopliae* on agar, or as a dried hyphal formulate or harvested spores.

The following are non-limiting examples demonstrating theoretical and functional parameters of the efficacy of the infection chamber, formulation and their components in controlling termites. In all cases termite behavior was observed to be significantly influenced by chamber components, and termite populations were significantly reduced by the fungus present in the infection chambers.

Example 1: Weed Wood Termites (*Zootermopsis angusticollis*) Directly Exposed to *Metarhizium* and *Beauveria* to Demonstrate Efficiency of the Fungi in killing termites.

Termites in groups of five were transferred to a 7 day old culture plate of growing fungus. After 5 minutes, they were moved to a clean plate, and excess conidia were removed. After 30 minutes, the termites were transferred to a holding plate lined with a layer of damp filter paper and Kim-wipes. The holding

plates were then placed in a plastic box with wet paper towels at room temperature. The results are shown in Table 1.

Table 1. Observations of Termites (*Zootermopsis agusticollis*) after Contact with Cultures of Selected Fungi

Fungal Agent	Termite Appearance 30 min. Post Exposure	Termite Mortality at day 3	Cadaver Appearance at day 5
M. anisopliae ATCC 62176	slow	100%	coated with fungus
B. bassiana ESC 71	sick	100%	coated with fungus
B. bassiana ARSEF 721	sick	80%	some fungal growth
Control	well	0%	not applicable

As shown in Table 1, after three days all of the #62176 and #71 treated termites were dead and all of the control termites were alive. After five days, cadavers showed coatings of fungal growth.

It can be concluded that direct contact of individual termites with pathogenic fungal cultures resulted in death of the termites.

Example 2: Infection of Eastern Subterranean Termites (*Reticulitermes flavipes*) by Direct Exposure of Termites to Fungal Isolates.

Wood containing termites was collected from a forest site in Montague, Massachusetts. The termites were separated from the wood and placed into laboratory colonies from which individuals could be removed for bioassay. Ten termites were placed for 15 minutes on the surface of a petri plate containing sporulating pathogenic fungus. After treatment, termites were transferred to a holding plate containing filter paper and observed over time.

It is apparent from the results shown in Table 2 that some strains of *Metarhizium* and *Beauveria* are very effective against termites, and can be used for termite control. Since soil isolates also showed high efficacy, this demonstrates that isolates of these pathogenic fungi could be obtained from soil using conventional methods.

Example 3: Infection of Colony Members By Release of Directly Infected Termites into a Colony

Termites (*Z. angusticollis*) in groups of four were placed in a petri dish with a small piece of wood (2 cm x 0.5 cm x 0.5) coated with a mixture of cellulose powder and either *M. anisopliae* or *B. bassiana* conidia scraped from an agar growth plate. Termites were exposed to this source of infection for one hour, then moved to a holding plate. After 48 hours, one of the exposed termites was introduced into another group of termites which had not been exposed to the fungus. Nine days after contact, one termite from the second group, which demonstrated no signs of illness, was transferred to a third group of unexposed termites. The mortalities of each of these groups of termites were observed over time.

Five days after the initial exposure to fungus, members of the first group of termites in both dishes were dead. Between days 13 and 15, the second group died. On day 23, 14 days from the last transfer, all exposed termites were dead and some were sprouting fungus. All of the control termites, in each of the groups, were alive.

The results of this study demonstrate that pathogenic fungus is transferred among termites, and results in the death of termites that never contacted the original infection source. Transfer of infection presumably occurred during the performance of social behaviors such as grooming.

Table 2: Fungi Active Against *Reticulitermis flavipes* by Contact Bioassay[1]

Source	Isolate #	Genus	Percent Mortality at 24 hrs	Percent Mortality at 72 hrs
ARSEF[2]	1112	Metarhizium	100	100
	1912		100	100
	2080		100	100
	925		60	100
	1095		60	100
	538		40	100
	1080		40	100
	1116		40	100
	2167		40	100
	23		10	100
	2951		0	100
	1489		0	100
	1878		0	80
ARSEF	252		90	100
	721		63.6	100
	1079		0	80
ATCC[3]	38249	Metarhizium	70	100
	62176		0	100
ATCC	48585	Beauveria	50	100
	48586		0	80
Soil ESC[4](JK)	130	Metarhizium	40	100
Soil ESC (JK)	131		30	100
Soil ESC (JK)	196		0	100
Insect ESC (JK)	10	Beauveria	90	100
Insect ESC	71		80	100
Insect ESC	70		60	100
Soil ESC (JK)	223		50	100
Insect ESC (JK)	241		30	100
Soil ESC (JK)	161		0	100

1. Contact bioassay: Animals were placed on a plate of growing fungus for 15 min. They were then transferred to a holding plant containing damp filter paper for observation.

2. ARSEF - USDA - ARS Collection of entomopathogenic fungal cultures.

3. ATCC - American Type Culture Collection.

4. ESC - EcoScience Corporation Isolate.

Example 4: Infection of T rmites by exposur t Fungus Outside of the Nest

Three small petri plates (15 x 100 mm) and one large plate (20 x 150 mm) were connected with tubes to form a series of interconnected chambers. The large plate had a water reservoir and a few pieces of wood to simulate a nest. Termites were allowed to live in this environment for a month. Fungal formulations of conidia, either of *M. anisopliae* or of *B. bassiana*, were applied as a coating on filter paper disks and/or toothpicks which were placed in the outermost chamber. There were six termites in each unit, and the treatments were repeated twice.

The results are shown in Figure 2. Data from the duplicates were combined to increase the sample population. Those treated with ATCC 62176 died with an LT_{50} of 26 days, while all of the controls were alive. Similar results occurred when the experiment was performed with ESC 71, which resulted in an LT_{50} of 22 days.

The conclusion from these studies is that all members of a small colony of termites can be killed by fungal infection when formulated fungus is placed within the foraging zone of the termites.

Example 5: Comparison of Fungal Formulations for Application to Soil to Infect Termites from a Site Distant from a Nest

1. <u>Soil Box Efficacy Tests</u>

The bottom of a plastic box (15 x 20 x 35 cm) was lined with 2 to 3 cm of soil. A simulated nest containing 300 termites was placed at one end of the box. The nest was built from two 20 x 100 mm petri dish bottoms, with four holes cut in the sides, placed open faces together, and filled with filter paper and wood fragments. Termites were allowed to adjust to the environment for 3 to 10 days. At the opposite end of the box, a hole 1.5-2 cm deep was dug in the soil,

and fungal formulation was applied. A wood block (5 cm x 3 cm x 7 cm) was placed in the hole containing formulation, and was half buried in the soil. The formulations consisted of (A) a granular form (oat bran cereal and hyphae) and (B) a powder form (cellulose and conidia). Wood, soil, and nest were inspected periodically for fungal activity against the termites.

The results shown in Table 3 demonstrate that wood can be protected by applying fungal formulation in the vicinity of the wood. It also demonstrates that a termite colony can be killed by the application of formulated fungus at the site of termite attack, which is at a distance from the nest.

Table 3. Termite Response to Formulated Fungus in Soil

Fungus	Fungal Growth and Termite Response	
	Day 3	Day 20
ATCC 62176		
Formulate A	Fungal Growth	Termites All Dead
Formulate B	No Growth	Termites All Dead
ESC71		
Formulate A	Fungal Growth	No Termite Around Wood, Nest Alive
Formulate B	No Growth	Termites Throughout, Nest Alive
Control		
Formulate A	Termite Tunnels Under Wood	Termites Throughout, Tunnels Over Wood
Formulate B	Termite Tunnels Under Wood	Termites Throughout, Tunnels Over Wood

Example 6: Screening for Fungal Pathog nicity Induced at a Distance from Termite Colonies.

The bottom of a plastic box was lined with 2 to 3 cm of soil. An artificial nest containing approximately 300 termites was placed at one end. The nest was built from two 20 x 100 mm petri dish bottoms, with four holes cut in the sides. The two petri dishes were placed open faces together, and filled with filter paper and wood fragments. Termites were transferred into the nest and allowed to adjust to the environment for three to seven days. At the opposite end of the box a hole was dug into the soil, dried hyphal formulation was applied, a wood block (5 x 3 x 7 cm) was placed on the hole, and soil was backfilled. Wood, soil, and nest were inspected periodically for fungal activity against the termites.

The efficacy of a group of isolates is shown in Table 4. In most cases, the fungus grew well. In specific cases termites were repelled from the area of fungal growth. In a limited number of cases, termites were found dead in the nest. Subsequently, fungi were recovered from cadavers from the nest.

The isolates which were most effective in killing termite nests were ATCC 62176 and ESC 70, a *Metarhizium* and a *Beauveria* respectively. Other highly effective and repellent isolates were 2080 and 1112.

Table 4. Response of *Reticulotermes* to Formulated Isolates in Soil Arenas.

Isolates	Termite Activity In Soil/On Wood	Termite Mortality in Nest at 30 Days
ATCC 62176 *Metarhizium*	Avoid Fungus, No tunnels	100%
ESC 70 *Beauveria*	Avoid Fungus, No tunnels	100%
ARSEF 2080 *Metarhizium*	Avoid Fungus, No tunnels	>90% dead, remainder sick
ARSEF 1112 *Metarhizium*	Reduced tunneling under wood, >20 found dead in soil	>90% dead
ESC(JK) 10 *Beauveria*	Reduced tunneling under wood, dead with fungus in soil	<10% dead in nest
ARSEF 252 *Beauveria*	Termites throughout area, >20 dead in soil	<10% dead in nest
ESC(JK)242 *Beauveria* restricted tunneling	Avoid fungus, in soil,nest	about 10% dead alive
ARSEF 721 *Beauveria*	Avoid fungus	Nest alive
ARSEF 1079 *Beauveria*	Reduced activity	Nest alive
CONTROL	Termites throughout area	Nest alive

Example 7: Tests f r T rmit Attractants.

Initial studies were conducted over short distances to determine the effectiveness of *G. trabeum* as an attractant. Arenas (60 x 60 x 5 cm), made of glass and wood, were coated with a thin layer of plaster. In the final experiment a thin layer of soil was put down on the top of the plaster. Termites (150 to 300) in an inverted petri dish, with six holes in the side and containing wet filter paper, were placed to one side of the arenas.

In one set of experiments, microcentrifuge tubes (1.5 ml) filled with (a) potential attractants and (b) control materials were placed 15 cm apart and 25 cm away from the nest at the other side of the arena. Arenas were videotaped by a time-lapse camera and observed for 15 days. In another set of experiments, a similar set up was used except that *G. trabeum* sawdust and a mixture of *M. anisopliae* (conidia or hyphae) and *G. trabeum* sawdust were used to fill the tubes instead of *G. trabeum* sawdust and control sawdust. All experiments were repeated three times.

Results show that *G. trabeum* infected sawdust is preferred over uninfected sawdust. When *M. anisopliae* conidia or hyphae are mixed with *G. trabeum*, termites do not react to the presence of *M. anisopliae;* that is, they enter both the attractant tube and the attractant plus *Metarhizium* tube. In both cases they contacted the tubes 2-7 hours after the experiment was set up and excavated the sawdust or the *G. trabeum/M. anisopliae* sawdust mixture in the tubes. When soil was present they built soil tunnels toward the test samples. In the test comparing *M. anisopliae* hyphae plus *G. trabeum* sawdust with *G. trabeum* sawdust alone,

the nest exposed to the *M. anisopliae* was completely dead (100% mortality) after 15 days. The conidial preparation of *M. anisopliae* with *G. trabeum* sawdust did not achieve this high degree of mortality.

The conclusions of these studies were that (1) termites preferred wood partially decayed by the fungus, *G. trabeum*, over non-decayed wood, and that the decayed wood initiates termite recruitment and excavating activity, and (2) in the presence of *G. trabeum*, termites ignored the lethal fungus and engaged in similar activities; that is to say the *G. trabeum* can overcome the repellency effect of the lethal fungus.

Example 8: Establishment of Termite Foraging Range using Attractant Fungus.

Arenas (122 x 366 x 30.5 cm) were set up and covered with 1 cm of soil. A double petri plate artificial nest, containing approximately 1000 termites and filter paper, was placed at one end of the arena 61 cm away from three of the four side walls.

A) *G. trabeum* decayed wood fragments (15 x 3 x 2 cm) were placed at distances of 122, 183 and 244 cm away from the nest in the center of the arena. These were left for one week, and were monitored on alternate days for termite activity.

B) Cardboard tubes (15 cm high x 4 cm diameter) filled with sawdust which had been inoculated with *G. trabeum* and incubated for several weeks were placed at a distance of 122, 183 and 244 cm from the nest. These were left for one week, and were observed periodically for termite activity.

C) Wood fragments and cardboard tubes, as above, were moved to within 60 cm of the nests; these

were left for one week, and periodically checked for termite activity.

Termites were observed building tunnels at 30 to 65 cm from the nest. Individuals were seen in areas up to 200 cm from the nest. However, no termites were observed on, in or around the wood fragments in experiment A or in the tubes in experiment B. On the other hand, as shown in experiment C, once the wood fragments or the cardboard tubes were moved closer toward the nest, termites were found in the wood pieces and the tubes overnight.

The range of termite foraging may be more limited under laboratory conditions than in the field. Recruitment under such conditions may occur only over a limited distance. Sufficient food in the nest may also reduce the need for foraging. However, even under such conditions, *G. trabeum* clearly acts as a recruitment stimulant.

Example 9: Attraction of Termites to Fungal Attractant Chambers.

A. Wood Test

Approximately 1000 termites were separated from wood collected from a forest area in Montague, Massachusetts, and put in a petri dish with wet filter paper.

The test arena was 122 x 122 x 30 cm (length x width x height), made of plexi-glass with no cover. The arena was filled with 5 cm of soil. Autoclaved wet wood fragments (15 x 3 x 2 cm) were inoculated with *G. trabeum*, grown for up to 8 weeks, and placed in a paper tube 4 cm in diameter and 13 cm in length. Autoclaved wood fragments were treated similarly but without the inoculation with *G. trabeum*. In the third case, non-autoclaved wood fragments were used to fill

the same kind of paper tubes. The termite colony was placed in the center of the arena covered with a petri dish. Four openings in the wall of the petri dish served as entrances for the termites in four directions, each pointing to a corner of the arena. The tubes were placed vertically against the arena walls three to a side, 10 cm apart, each partially inserted in the soil. The arrangement of the tubes was as follows:

Side 1:	Tube A - *G. trabeum* wood
	Tube B - autoclaved wood
	Tube C - wood
Side 2:	Tube D - autoclaved wood
	Tube E - wood
	Tube F - *G. trabeum* wood
Side 3:	Tube G - *G. trabeum* wood
	Tube H - autoclaved wood
	Tube I - wood
side 4:	Tube J - autoclaved wood
	Tube K - *G. trabeum* wood
	Tube L - wood

After one day, no termites were found in any of the tubes, though termites in the nest did invade the soil. Five days later, termites were found in all tubes containing *G. trabeum* but in no other tubes.

The conclusion to be drawn from this experiment is that *G. trabeum* inside a chamber is effective in attracting termites even in the presence of soil.

B. Sawdust Test

Experimental conditions were as described above for the wood test except as noted. Various kinds of wet sawdust, listed as follows, were substituted for the wood fragments in the cardboard tubes: (1) non-autoclaved fresh sawdust (moistened); (2) autoclaved sawdust (moistened and autoclaved for 25 minutes); (3) *G. trabeum* sawdust (moistened and autoclaved, then

inoculated with *G. trabeum* and incubated at room temperature for about 8 weeks).

The testing tubes were arranged as described above in the following order:

Side 1: Tube A - autoclaved sawdust
Tube B - non-autoclaved sawdust
Tube C - *G. trabeum* sawdust
Side 2: Tube D - non-autoclaved sawdust
Tube E - autoclaved sawdust
Tube F - *G. trabeum* sawdust
Side 3: Tube G - non-autoclaved sawdust
Tube H - autoclaved sawdust
Tube I - *G. trabeum* sawdust
Side 4: Tube J - autoclaved sawdust
Tube K - *G. trabeum* sawdust
Tube L - non-autoclaved sawdust

The results of this experiment were that termites in the colony went down into the soil, leaving few in the nest. Termites were found in all the *G. trabeum* tubes and in one autoclaved tube (Tube H). The conclusion from this experiment was that *G. trabeum* attracts termites, but that sawdust alone does not in the time frame and distances used in this study. Termites chose only *G. trabeum* inoculated chambers from a group of chambers placed in the soil.

Example 10: Comparison of Fungal Formulations in Soil Box Choice Test.

The bottom of a plastic box was lined with 3 cm of top soil. An artificial nest containing 300 termites was placed at one end. The nest was built from two 20 x 100 cm petri dish bottoms, with four holes cut in the sides, placed open faces together, and filled with filter paper and wood pieces. Termites were allowed to adjust to the environment for 3 to 10 days. Two small blocks of wood were placed in shallow holes dug in the soil on the other side of the box.

Each block was treated with either attractant (*G. trabeum* infected wood fragments), or formulated fungus (ATCC 62176 hyphae mixed with oat bran), or both or untreated. The arrangement of blocks in each box is shown below:

Box	Block 1	Block 2
A	wood	wood and attractant
B	wood	wood and formulated 62176
C	wood and formulated 62176	wood and attractant
D	wood	wood and attractant and formulated 62176

The attractant was put on top of the wood block while the formulated lethal fungus (62176) was placed in the soil under the wood block.

When given a choice (in box A), termites preferred wood associated with *G. trabeum* over wood alone. Termites built visible tunnels over the surface of the wood block to reach *G. trabeum* infected wood. In box B, termites preferred the untreated wood block over the wood block placed on a *M. anisopliae* formulation. These results indicate that *M. anisopliae* repels termites. In box C, termites preferred the *G. trabeum* treated wood block over the wood block placed on a *M. anisopliae* formulation. When *M. anisopliae* and *G. trabeum* were placed around the same block (box D), the termite nest appeared to be killed faster than *M. anisopliae* alone as in box B.

G. trabeum was shown to attract and cause termites to recruit others, to build and feed. *M. anisopliae*, when encountered by termites, repels the termites. When the two fungi were combined, the termite contact with the lethal fungus was facilitated by the presence of the attractant (recruitment

stimulant). Therefore, one may conclude that combined in proper proportion and spacial configuration, the *G. trabeum* will overcome the repellent nature of *M. anisopliae* and ultimately lead to extensive infection and demise of the colony.

Example 11: Repellent Effect of *M. anisopliae* ATCC 62176 granular formulation.

In previously described arenas, two petri dishes containing *G. trabeum* sawdust were placed at a distance of 45 cm away from each other and 60 cm away from the point where the termite colony was subsequently placed. A 20 mm wide ring of culture 62176 formulation was placed around one petri dish while the other petri dish was surrounded by the control formulation (oat bran without *M. anisopliae*). The formulations were then mixed with the soil to form a Formulation Zone around the *G. trabeum* sawdust dishes. The arena was then sprayed with water to set the soil as well as the formulations. Five days later, a termite colony was transferred into the arena 60 cm away from both dishes. Fungal growth and termite activities inside the *G. trabeum* dishes were observed and used to evaluate the repellent effects of the formulations. Three arenas, each with the same set up as described above, were set up at the same time as replicates I, II and III.

Twelve days after the termite colonies were introduced, termites had passed through the Control Formulation Zone and reached the *G. trabeum* dish in all three arenas. There were several hundred termites in each of these three dishes. Termites inside the dishes also excavated the sawdust and built tunnels, which were visible from outside of the dish. In the same period, a few termites passed through the 62176

Formulation Zone in only two arenas (arena I and II) and no more than 20 termites were found in either of these attractants dishes. No termite activities nor any tunnels were visible from outside of the petri dish surrounded by 62176.

It is clear that there was a significant difference between the number of termites passing through the 62176 Formulation Zone and the number of termites passing through the Control Formulation Zone. This indicates that the 62176 had a deterrent effect on termites provided that this formulation is spatially separated from the *G. trabeum* sawdust.

Example 12: Use of *M. Anisopliae* ATCC 62176 as a Granular Formulation to Protect a Wood Structure in an Arena.

Materials and Methods

The arena was a 122 x 366 x 30.5 cm (wide x long x high) Plexi-glass arena with 15 mm depth of soil on the bottom.

Termites were collected from Montague, Massachusetts and kept at room temperature. Prior to use, termites were removed from the wood and transferred to a petri dish filled with filter paper.

The wood frames were constructed from stock lumber.

Part A was made of 4 pieces of 5 x 10 x 30 cm wood with numerous 2 cm deep grooves on the surfaces. These 4 pieces of wood were nailed together and formed a 10 x 35 x 35 cm wood rectangle.

Part B was made from 4 pieces of 5 x 10 x 30 cm wood. They were nailed together to form a 5 x 40 x 40 cm wood rectangle. Part B was nailed on top of Part A to form the wood frame.

ATCC culture 62176 mycelium harvested from liquid culture was mixed with sterilized oat bran flour. The mixture was dried by air flow at room temperature overnight then ground and sized through a #14 sieve to form granules.

G. trabeum wood was prepared by autoclaving wood fragments (13 x 3 x 2 cm), inoculating them with *G. trabeum*, and incubating them at room temperature for 4 to 8 weeks.

The wood frame was placed in the center of the arena. Around and under the foundation of the wood frame, culture ATCC 62176 formulation was mixed with soil and the wood frame was set on top. In the center of the wood frame, a pile of *G. trabeum* wood was set on the soil. The wood frame was then covered with a piece of cardboard to reduce drying and keep the area dark. Into each end of the arena 120 cm away from the wood frame one termite colony (600-1000 individuals) was transferred together with a few pieces of wet filter paper. The colony was then covered with a petri dish which had a few openings on the side to form termite nests. Of the two termite colonies used, one had about 1000 termites while the other had about 600 termites. Each arena was then examined once or twice a week for fungal growth as well as termite activities in the wood frame and in the *G. trabeum* wood pile, and sprayed with water periodically to keep soil moist. Another arena was set up with similar materials except that a control formulation (oat formulation without 62176 mycelium) and two termite colonies, each containing 1000 termites, were used.

Two days after the formulation was applied, fungal growth was observed on the formulation. One

week after the application, 62176 started to conidiate. After the beginning of conidial production, the formulation, and conidia were visible for about 20 days. During the same period, termites were found living inside the nests as well as inside soil around the nests, but not in the protected "wood frame" nor in the "*G. trabeum* wood pile."

Five weeks after the experiment was set up, several hundred termites were found under the "wood frame" and inside the "*G. trabeum* wood pile." Ten days later, all termites (a few hundred) under the wood frame were found to be dead while termites inside the "*G. trabeum* wood pile" were still alive.

Two weeks after the termites moved into the "*G. trabeum* wood pile" and the "wood frame", dead termites were found inside the "*G. trabeum* wood pile" and in one of the termite nests (the nest originally had about 1000 termites). Fungus was found growing out of most of the termite cadavers in the soil. *M. anisopliae* was isolated from cadavers recovered from the nest.

Upon the termination of the experiment (67 days after the experiment was set up), the termite nest and soil in the arena as well as the "wood frame" were taken apart and examined thoroughly. Less than 10% of the termite population in one nest (the one where dead termites were found) were alive, i.e., more than 90% of the termites in this nest were killed. In the other nest, no dead termites were found and the number of termites recovered from the nest and soil approximated the original termite number. More importantly, the wooden structure had no visible damage from termite attack.

In a control experiment where the formulation used at the structure contained no *M. anisopliae*, termites were found at the wood structure after one week. Subsequently, they tunneled up, around, and into the wood. Several hundred termites, including secondary reproductives and eggs, were found living inside the wood frame.

It appears that the interaction between termite and fungus occurred in three phases. First, it took colony members one week to forage across the 120 cm to encounter the structure. Next the termites at the treated structure recognized the presence of the *Metarhizium* and were repelled. Once the fungus reached its maximum growth in the soil, it started to die back. The repellent quality was soon lost and the assaulting termites crossed the barrier. However, the recently produced conidia were still present in the soil. When the foraging termites passed through the area applied with fungal formulation, the conidia attached to the termites and infected them. The foragers also brought conidia back to the nest to infect other colony members. The action is seemingly rapid enough to protect the structure even when termite numbers are moderately high.

Example 13: Soil Arena Test of Prototype *M. anisopliae* ATCC 62176/*G. trabeum* ATCC 13021 Impregnated Chambers.

Chambers: Paper tubes (3 cm diameter x 5 cm high) were filled with a uniform mixture of *G. trabeum* sawdust, *M. anisopliae* ATCC 62176 Formulation and 62176 conidia in the ratio 100:5:1.

Blank Control Chambers: Paper tubes filled with *G. trabeum* sawdust and granular at a ratio of 100:5.

The arena (122 x 122 x 30) and termites were the same as in Example 9. Each petri dish contained about 1000 termites and was treated as a colony. Arenas were filled with soil to a depth of 5 cm.

In each arena, there was one termite colony in the center of the arena and four infection chambers. Each chamber was placed against and in the center of each arena wall and buried in soil. Three arenas were set up with *M. anisopliae* ATCC 62176/*G. trabeum* ATCC 13021 chambers as replicates and one arena with control chambers as control.

In all 62176 treated arenas, termites were found inside two of the four chambers one week after the test was initiated. However, two to three weeks later no termites could be found inside the chambers although there were still termites alive in the soil. In the control arena, termites were found inside one of the chambers from day 30 until the experiment ended on day 70.

In order to determine termite mortality in these soil arenas, two 62716 treated arenas were taken apart at day 45. In both arenas 30% to 40% of termite mortality was achieved. By day 70, 100% mortality was achieved in the third 62176 chamber treated arena while in the control arena no termite number reduction was found. It was also observed that in the control arena there were secondary reproductives, eggs as well as early instar nymphs.

It could be concluded from this experiment that *M. anisopliae* ATCC 62176 and *G. trabeum* ATCC 13021 impregnated sawdust chambers were able to attract and subsequently eliminate the termite population.

We Claim:

1. A composition for killing termites comprising an entomopathogenic fungus for termites in combination with an effective amount of a recruitment stimulus to overcome the repellency of the entomopathogenic fungus for the termites.

2. The composition of claim 1 wherein the recruitment stimulus for termites is *Gloeophyllum trabeum*.

3. The composition of claim 2 wherein the *Gloeophyllum* is a fungal culture grown on a nutrient medium.

4. The composition of claim 1 wherein the recruitment stimulus for termites is a chemical composition produced by wood-decaying fungi.

5. The composition of claim 1 further comprising a housing for the entomopathogenic fungus.

6. The composition of claim 6 wherein the housing comprises a material selected from the group consisting of wood materials, cellulose based products, and synthetic plastics.

7. The composition of claim 6 further comprising a sheath for the housing.

8. The composition of claim 7 placed in a degradation-resistant sheath formed of a material selected from the group consisting of plastic, metal and wood.

9. The composition of claim 7 wherein the sheath is biodegradable.

10. The composition of claim 1 wherein the entomopathogenic fungus is selected from the group consisting of *Metarhizium*, *Beauveria*, *Entomophthora*,

Conidiobolus, *Paecilomyces Verticillium*, and *Absidia* species.

11. The composition of Claim 10 wherein the fungus is selected from the group consisting of *Metarhizium* and *Beauveria*.

12. The composition of claim 1 wherein the composition is in the form of a formulation containing fungal hyphae mixed with a cellulose based food product.

13. A method for attracting termites comprising providing at a site termites are to be attracted to *Gloeophyllum trabeum* .

14. The method of claim 13 wherein the *Gloeophyllum* is provided as a live culture.

15. The method of claim 13 wherein the *Gloeophyllum* is provided as an extract of a fungal culture.

16. The method of claim 13 further comprising providing in combination with the *Gloeophyllum* an entomopathogenic fungus for termites.

17. The method of claim 16 wherein the entomopathogenic fungus is repellant to termites.

18. The method of claim 17 wherein the entomopathogenic fungus is selected from the group consisting of *Metarhizium*, *Beauveria*, *Verticillium*, *Entomophthora*, *Conidiobolus*, *Paecilomyces* and *Absidia* species.

19. The method of claim 18 wherein the entomopathogenic fungus is selected from the group consisting of *Metarhizium anisopliae* and *Beauveria bassiana*.

20. The method of claim 16 further comprising a housing for the fungus.

21. The method of claim 20 wherein the housing comprises a material selected from the group consisting of wood materials, cellulose based products, and synthetic plastics.

22. The method of claim 20 further comprising a sheath for the housing.

23. The method of claim 13 further comprising placing the entomopathogenic fungus in combination with attractant at a site to be protected from attack by termites.

24. The method of claim 13 comprising providing a formulation containing fungal hyphae mixed with a cellulose based food product at the site where the structure is to be protected.

25. A method for repelling termites from a structure to be protected from termite attack comprising providing a fungus selected from the group consisting of entomopathogenic and repellent fungus at the site of the structure.

26. The method of claim 25 wherein the fungus is selected from the group consisting of *Metarhizium*, *Beauveria*, *Entomophthora*, *Verticillium*, *Conidiobolus*, *Paecilomyces* and *Absidia* species.

27. The method of claim 26 wherein the fungus is *Metarhizium*.

28. The method of claim 25 wherein the fungus is prepared in a formulation to mix into the soil.

29. The method of claim 25 wherein the repellent is an extract of cultures of entomopathogenic or other fungi.

30. The method of claim 28 further comprising placing the formulation in the chambers of a termite colony.

31. The method of claim 25 wherein the fungal culture is placed in a housing which is inserted into the soil around the structure to be protected.

32. A formulation to repel or kill termites comprising a fungus selected from the group of fungus lethally infecting termites and fungus repelling termites in combination with a cellulose based composition wherein the fungus is in a form selected for the group consisting spores, mycelium and combination thereof.

33. The formulation of claim 32 wherein the fungus is selected from the group consisting of *Metarhizium*, *Beauveria*, *Entomophthora*, *Conidiobolus*, *Paecilomyces* and *Absidia* species.

34. The formulation of claim 33 wherein the fungus is *Metarhizium* and the fungus is in the form of mycelium.

35. The formulation of claim 33 wherein the fungus is *Beauveria*.

36. The formulation of claim 33 wherein the fungus is obtained by screening soil isolates for their effect on termites.

37. The formulation of claim 32 wherein the cellulose based composition is selected from the group consisting of bran, cereal grains, and sawdust.

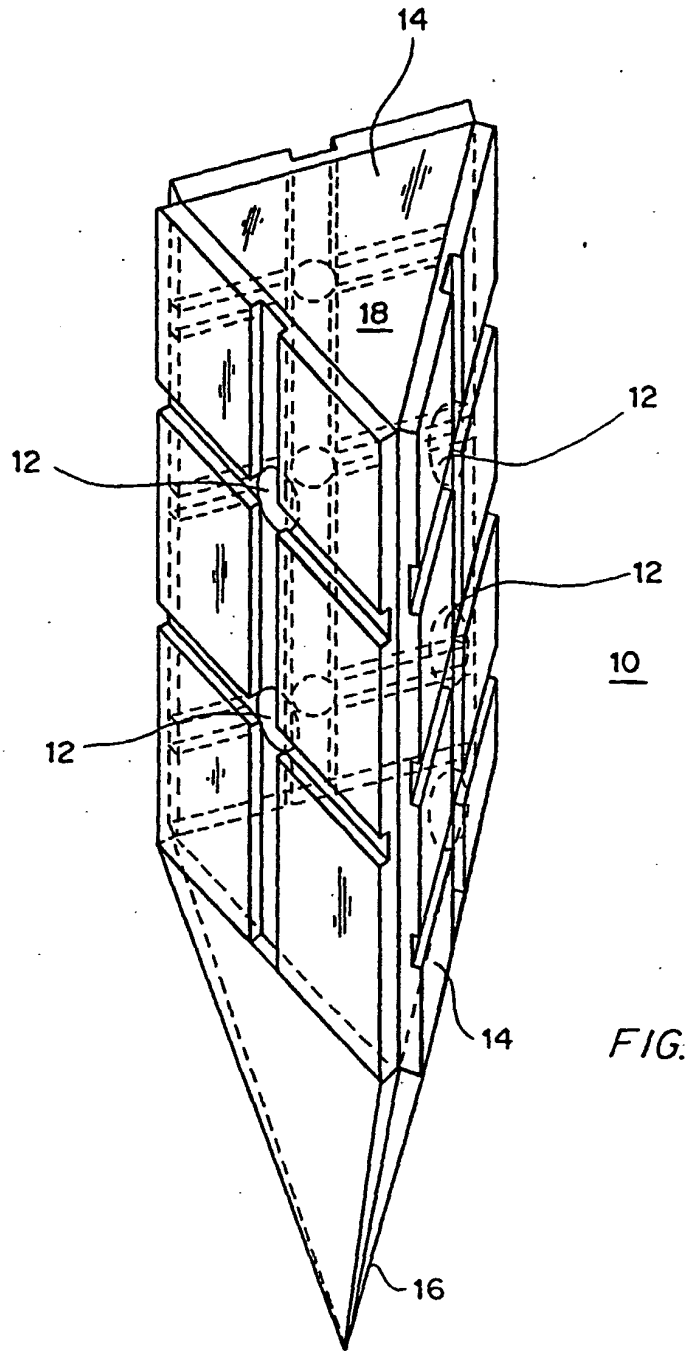
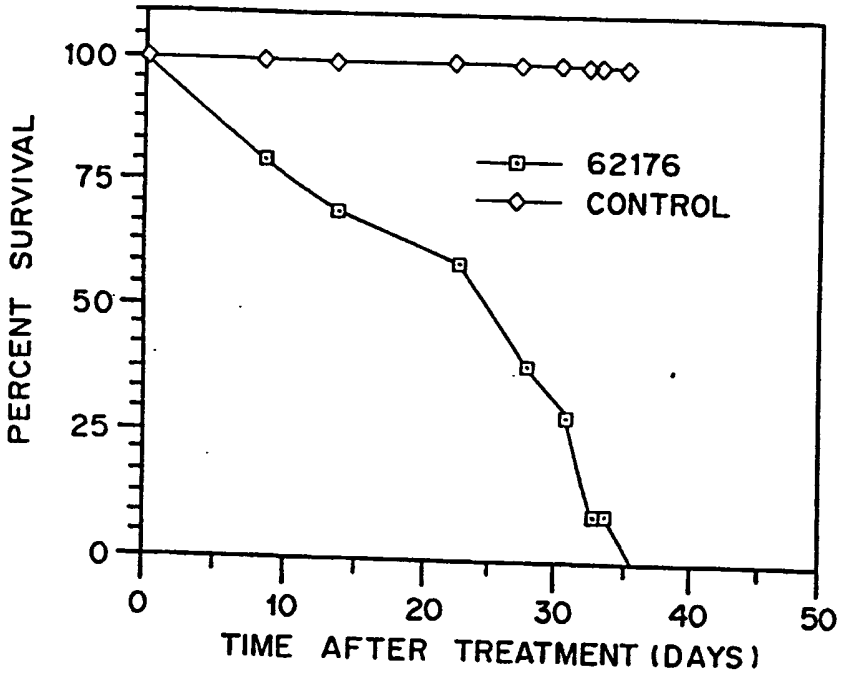


FIG. 1


100
75
50
25
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PERCENT SURVIVAL
62176
CONTROL
0
10
20
30
40
50
TIME AFTER TREATMENT (DAYS)

FIG. 2a

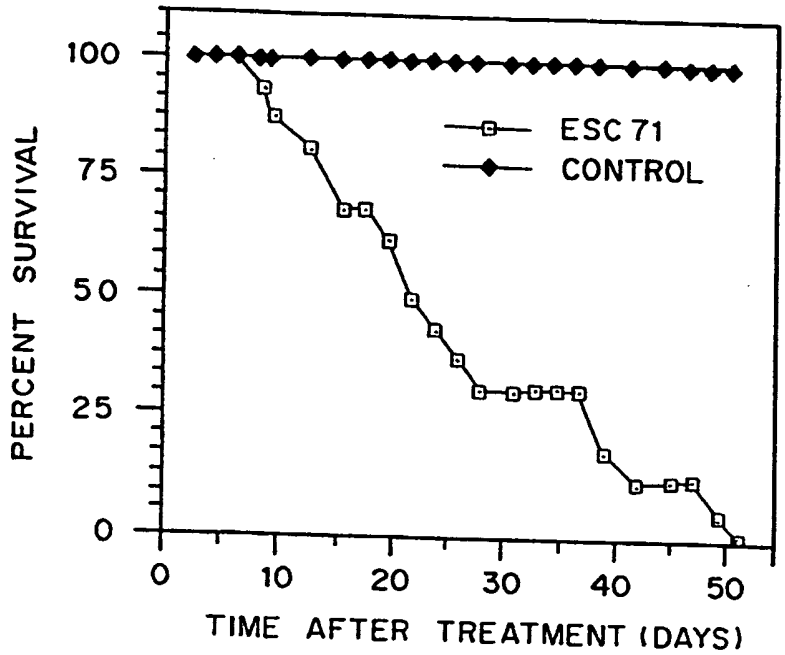
100
75
50
25
0
PERCENT SURVIVAL
ESC 71
CONTROL
0
10
20
30
40
50
TIME AFTER TREATMENT (DAYS)

FIG. 2b

INTERNATIONAL SEARCH REPORT

Intern. al Application No
PCT/US 93/07143

A. CLASSIFICATION F SUBJECT MATTER
IPC 5 A01N63/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO,A,93 09672 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION) 27 May 1993 see page 6, last paragraph - page 8, paragraph 2 ---	1-37
X	FR,A,1 533 177 (INSTITUT PASTEUR) 19 July 1968 see claims	1,5-12, 32-37
Y		2-4, 16-24
	--- -/---	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 November 1993	30. 11. 93

Name and mailing address of the ISA	Authorized officer
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Intern. al Application No
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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,3 070 495 (G.R. ESENTHER ET AL.) 25 December 1962 see c lumn 1, line 49 - line 57 see column 2, line 10 - line 12 see claims	13-15
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